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(54) **METHOD FOR DETECTING BACTERIAL ENDOSPORES IN A SEALED CONTAINER**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 783 days.

This patent is subject to a terminal disclaimer.

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C12Q 1/24 (2006.01)

(52) **U.S. Cl.** **435/30**; 435/808; 436/48; 436/52; 436/177

(58) **Field of Classification Search** 436/48, 436/52, 177; 73/863.51, 863.71, 863.91; 435/287.2, 30, 808; *C12Q 1/24*
See application file for complete search history.

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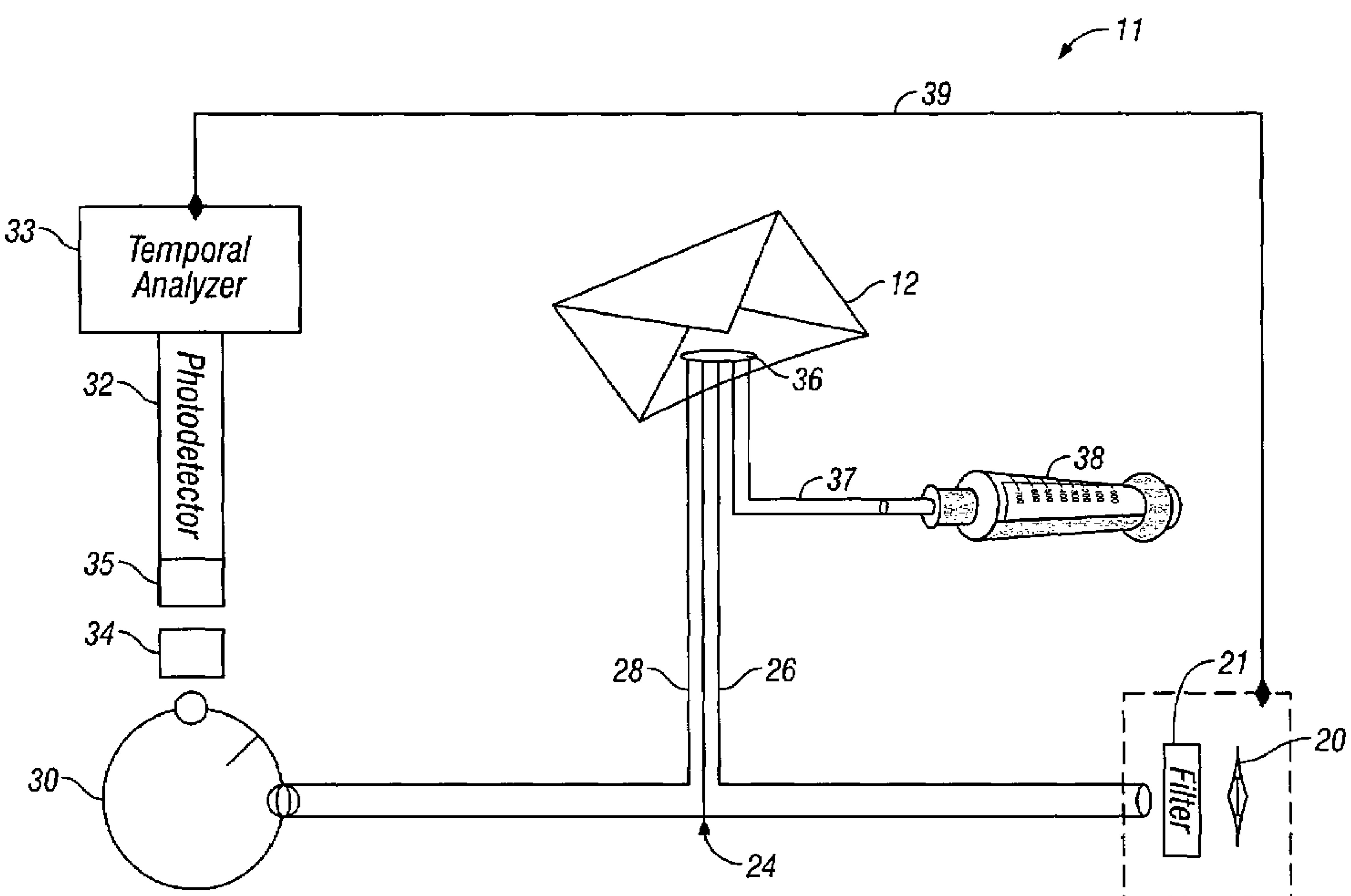
(57) **ABSTRACT**

A device for detecting bacterial endospores in a sealed container. The device has a suction tube connected to an aerosol concentrator containing a lanthanide salt solution, a suction pump engaged to the aerosol concentrator, an excitation energy source and an optical set-up for directing the excitation energy source to the lanthanide salt solution and collecting photoluminescence generated by the excited lanthanide salt solution. A method for detecting bacterial endospores is also provided.

7 Claims, 2 Drawing Sheets



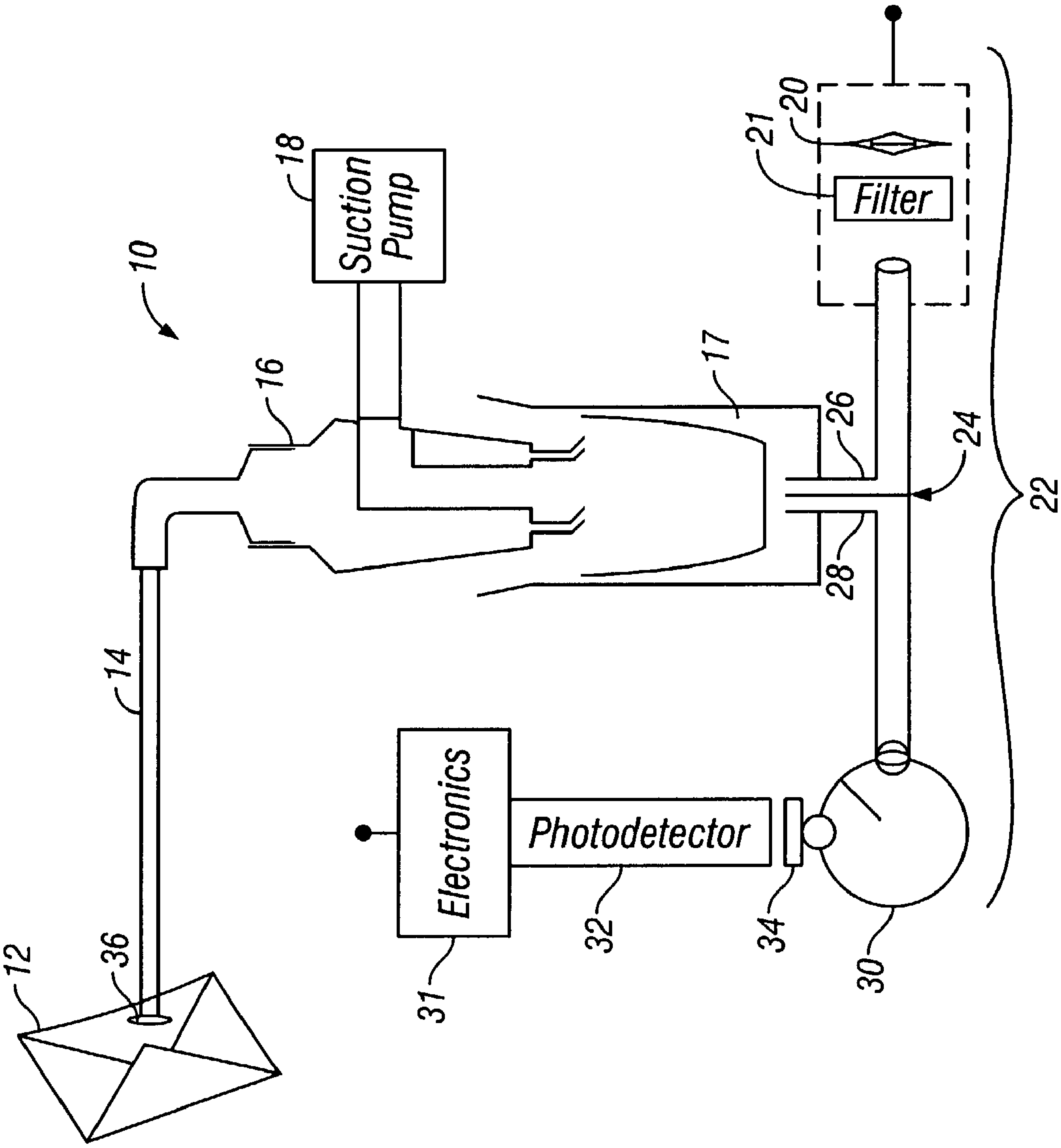

10
12
14
16
17
18
Suction Pump
20
21
Filter
22
24
26
28
30
31
Electronics
32
Photodetector
34
36

FIG. 1

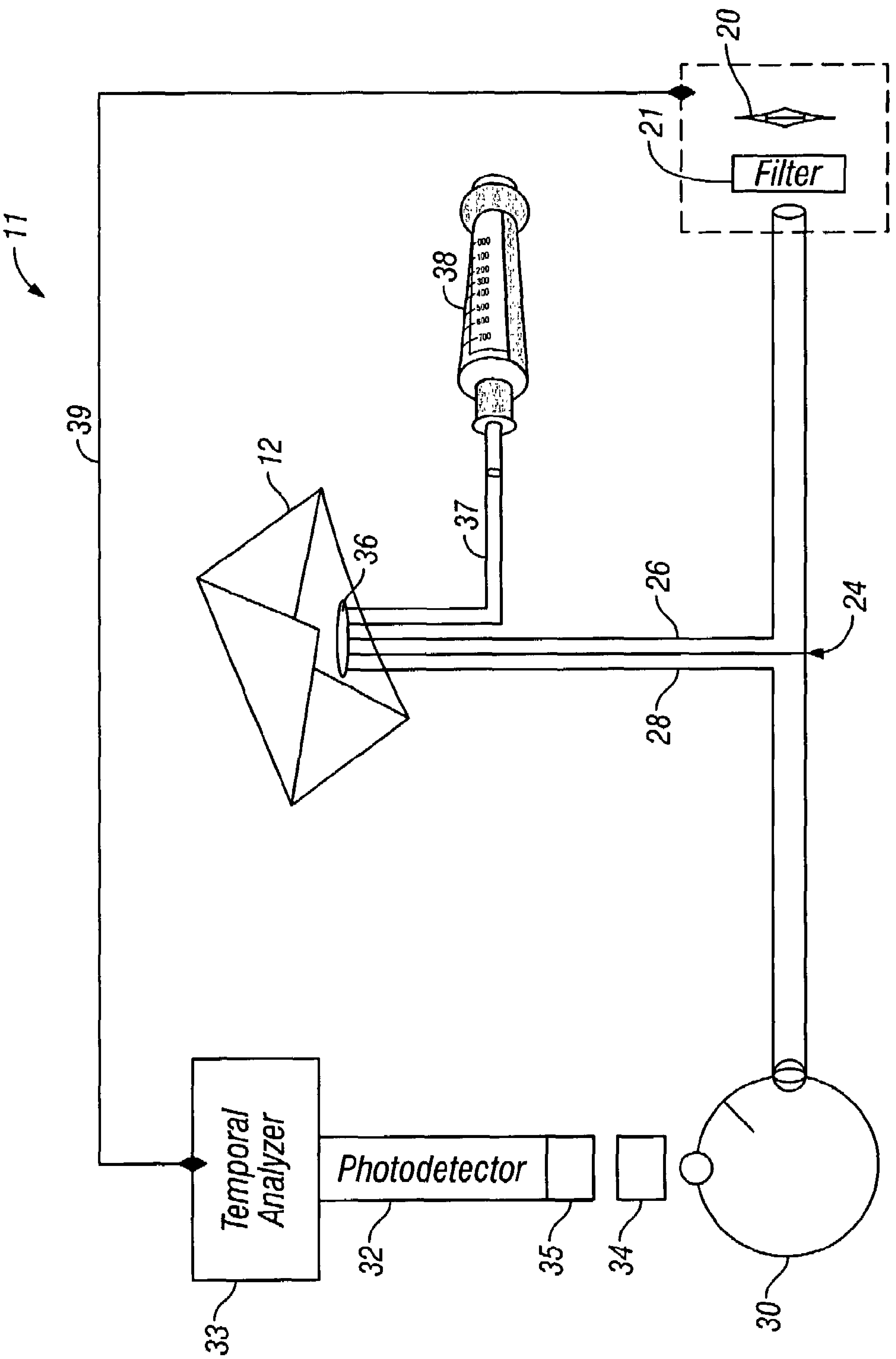

11
20
21
Filter
38
39
12
37
36
26
24
28
Temporal Analyzer
Photodetector
33
32
35
34
30

FIG. 2

METHOD FOR DETECTING BACTERIAL ENDOSPORES IN A SEALED CONTAINER

STATEMENT OF GOVERNMENT INTEREST

The invention described herein may be manufactured, used and/or licensed by or for the Government of the United States of America.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to particle detection and, more particularly, to detecting the presence of bacterial endospores in a sealed container.

2. Description of the Related Art

Recently, mail service within the United States has been disrupted by the spread of anthrax. Such mail service includes the United States Postal Service as well as private carriers such as Federal Express, United Parcel Service (UPS), DHL and the like. Anthrax is spread through bacterial endospores shipped through the mail or other delivery services and strikes its victim, intended or otherwise, once the mail is opened. This is because after the mail is opened, bacterial endospores are dispersed into the air where they can infect the victim either through inhalation or by contacting skin. In addition to anthrax, a bioterrorist can also spread endospores that cause other diseases such as botulism, tetanus and gas gangrene through the postal service.

Infected mail is very dangerous. To date, people who have opened the mail and some postal workers who have merely sorted unopened mail have become infected by means that are still unknown. Opened mail, however, is the most dangerous in that once the mail is opened, small air currents immediately disperse the endospores. This coupled with privacy issues encountered when handling the mail make it desirable to detect the endospores without having to actually open the mail.

Known tests for detecting bacterial contamination are usually slow and often unreliable. Serological methods use antibodies which often have large cross reactivities that can cause false alarms. Mass spectroscopy has extremely complex spectra that are difficult to analyze and could cause false alarms. DNA testing is extremely slow and expensive.

In order to be an effective bioagent, endospores must be concentrated. Because bacterial endospores are not commonly found in nature, their ambient concentration is extremely low. As a result, the concentration of these endospores in poisoned mail is very high and a large quantity of endospores in a piece of mail is very likely to represent a bioattack involving anthrax, tetanus, gas gangrene or botulism. Therefore, a device and method that allows for the fast and specific detection of endospores without opening the mail or, at the very least, that only creates a small puncture or cut in the mail that can be rapidly sealed so that the bacteria either never leave the mail or are deposited in an aerosol concentrator would be an important improvement in the art.

SUMMARY OF THE INVENTION

A device for detecting bacterial endospores in a sealed container is provided. The device has a suction tube connected to an aerosol concentrator containing a lanthanide salt solution. A suction pump is engaged to the aerosol concentrator and an optical set-up directs the excitation energy source to the lanthanide salt solution and collects photoluminescence generated by the excited lanthanide salt solution.

A method for detecting bacterial endospores is also provided in which the sealed container is punctured. A suction tube connected to an aerosol concentrator is inserted into the puncture. The step of drawing air from inside the container through the puncture and into the aerosol concentrator is performed. The air drawn from the container is deposited into a lanthanide salt solution and the salt solution is excited with an excitation energy source. Emissions from at least one lanthanide salt emission band are collected and photoluminescence in the emissions from the lanthanide salt in the solution are measured.

A device for detecting bacterial endospores in a sealed container in which a lanthanide salt solution capable of being injected into the sealed container is provided. The device has a set of optical fibers that detects an excitation energy source to the lanthanide salt solution and collects photoluminescence emissions generated by the excited lanthanide salt.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a schematic showing a device for the detection of endospores.

FIG. **2** is a schematic showing an alternative device for injecting a lanthanide salt solution into a sealed container in order to detect endospores in the container.

DETAILED DESCRIPTION

A device for detecting bacterial endospores in a sealed container is provided. The device is comprised of a suction tube connected to an aerosol concentrator containing a lanthanide salt solution, a suction pump engaged to the aerosol concentrator, an excitation energy source and an optical set-up which directs the excitation energy source to the lanthanide salt solution and collects photoluminescence generated by the excited lanthanide salt solution.

A method is provided for detecting bacterial endospores inside a sealed container, with the method being comprised of the steps of puncturing the sealed container to form a puncture, inserting a suction tube connected to an aerosol concentrator into the puncture, drawing air from inside the container through the puncture and into the aerosol concentrator, depositing the air drawn from the container into a lanthanide salt solution, exciting the lanthanide salt solution with an excitation energy source, collecting emissions from a lanthanide salt emission band and measuring the photoluminescence in the emissions from the lanthanide salt in the solution.

In another embodiment, a lanthanide salt solution is injected through a puncture into the sealed container. Following the injection of the lanthanide salt solution, two sets of optical fibers are inserted into the container. Once this is done, the solution is excited with an ultraviolet light using one of the two sets of optical fibers. A second of the two sets of optical fibers is then used to collect photoluminescence emitted from the excited lanthanide salt solution. The collected photoluminescence are then measured to determine presence of bacterial endospores.

A device is also provided for detecting bacterial endospores in a sealed container where the device is comprised of a lanthanide salt solution capable of being injected into the sealed container, an excitation energy source and a set of optical fibers for directing the excitation energy source to the lanthanide salt solution and collecting photoluminescence emissions generated by the excited lanthanide salt.

A device **10** for detecting bacterial endospores in a sealed container **12** is provided. As shown in FIG. **1**, the device **10** is comprised of a suction tube **14** connected to an aerosol concentrator **16** containing a lanthanide salt solution, a suction pump **18** engaged to the aerosol concentrator **16**, an excitation energy source **20** and an optical set-up **22** for directing the excitation energy source **20** to the lanthanide salt solution and collecting photoluminescence generated by the excited lanthanide salt solution.

The aerosol concentrator **16** used in conjunction with the device may selectively be a bubbler, impinger or an impactor-type concentrator. Furthermore, the excitation energy source **20** can be, among other things, an ultraviolet light, a pulsed xenon arc lamp, a pulsed laser or modulated light source. The excitation energy source may be used in conjunction with a filter **21**, such as a broadband ultraviolet filter.

The optical set-up **22** seen in the example of FIG. **1** has a probe **24** having a first and a second optical fiber **26**, **28** wherein the first optical fiber **26** excites the lanthanide salt solution with an ultraviolet light and a second optical fiber **28** collects an emission from one of the lanthanide emission bands generated by the lanthanide salt solution upon receipt of the excitation energy source. These emission may then be collected in an integrating sphere **30** where they can be measured using a photodetector **32** such as a photomultiplier with associated analyzing electronics **31**. If necessary, a filter **34** (such as a narrow band filter at 540 nm) may be positioned between the integrating sphere **30** and the photomultiplier **32**.

The lanthanide salt can be in various forms including, but not limited to, europium chloride and terbium chloride. The sealed container **12** may be, among other things, an envelope, a box and a package.

Suction tube **14** used to draw an air sample may selectively contain a sealing apparatus capable of sealing a small opening puncture **36** created when the sealed container **12** is punctured. The sealed container **12** may selectively be placed under a protective device such as a hood such that if the sealed container is opened up even a small amount, the contents will not be freely dispersed into the environment. Alternatively, the sealed container **12** may selectively be placed in a safety container such as a plastic bag having a plug that allows the probe **24** of the optical set-up **22** to be inserted into the sealed container **12**. The safety container plug may selectively be resealed after examination. A plug may alternatively be placed at the end of an air sampler to allow the container to be resealed after examination.

The embodiment of FIG. **1** shows a device **10** where air is drawn from a puncture **36** in the container **12** into an aerosol concentrator **16** that contains a terbium chloride ($TbCl_3$) solution **17**, and photoluminescence from terbium dipicolinate is measured in the concentrator. The device **10** removes air from a sealed container **12** and detects dipicolinic acid that comes from bacterial endospores. Once a puncture is made in the container **12**, a tube **14** for air is inserted in the puncture. Air is drawn into the aerosol concentrator **16** which deposits the aerosol into a solution of terbium chloride **17**. Probe **24** comprising two optical fibers **26**, **28** excites the solution **17** with ultraviolet light. Optical fiber **28** collects emission from one of the terbium emission bands (for example a band may be at 540 nm). The photoluminescence is measured after a short delay relative to the excitation pulse. Bacterial endospores may be immediately detected inside the aerosol concentrator. The detected endospores may be available for serological or DNA analysis.

A method for detecting bacterial endospores inside a sealed container **12** is also provided where the method is comprises of the steps of: (a) puncturing the sealed container **12** to form a puncture **36**; (b) inserting a suction tube **14** connected to an aerosol concentrator **16** into the puncture **36**; (c) drawing air from inside the container **12** through the puncture **36** and into the aerosol concentrator **16**; (d) depositing the air drawn from the container **12** into a lanthanide salt solution; (e) exciting the lanthanide salt solution with an excitation energy source **20**; (f) collecting emissions from at least one lanthanide salt emission band; and (g) measuring the photoluminescence in the emissions from the lanthanide salt in the solution.

In one embodiment of the method, the excitation energy source **20** is an ultraviolet light. The ultraviolet light may be transmitted by, among other things, a modulated light source, a pulsed xenon arc lamp and a pulsed laser. The lanthanide salt solution used may be in several forms including, but not limited to, terbium chloride and europium chloride.

The lanthanide salt solution may selectively be excited with an ultraviolet light using a first optical fiber **26** of a probe **24** having at least two optical fibers **26**, **28**. In this embodiment, second optical fiber **28** collects emission from one of the lanthanide salt solution emission bands. The sealed container **12** used may be any type including, but not limited to, an envelope, a box and a package.

A method is provided for detecting bacterial endospores inside a sealed container **12** where a lanthanide salt solution is injected into the sealed container **12** through the puncture **36**. Following the injection of the lanthanide solution, two sets of optical fibers **26**, **28** are inserted into the container **12**. Once this is done the lanthanide solution is excited with an ultraviolet light **20** using one of the two sets of optical fibers **26**, **28**. The photoluminescence emitted from the excited lanthanide salt solution is then collected using the second of the two sets of optical fibers **26**, **28**. The collected photoluminescence is then measured to detect the presence of dipicolinic acid.

Again, the lanthanide salt used in the method can be, among other things, terbium chloride or europium chloride. Also, the light is transmitted by one of several sources **20** including, but not limited to, a modulated light source, a pulsed xenon arc lamp and a pulsed laser.

The solution is excited with ultraviolet light using a first optical fiber **26** of a probe **24** having at least two optical fibers **26**, **28**. In this embodiment, a second optical fiber **28** collects emission from one of the lanthanide salt solution emission bands. As mentioned above, the sealed container **12** can be in several forms, including an envelope, a box and a package.

An alternative embodiment is provided in FIG. **2** for detecting bacterial endospores in a sealed container **12** with a device **11** utilizing a lanthanide salt solution capable of being injected into the sealed container **12**. The device comprising an excitation energy source and a set of optical fibers **26**, **28** for directing the excitation energy source **20** to the lanthanide salt solution and collecting photoluminescence emissions generated by the excited lanthanide salt. A broadband ultraviolet filter **21** may selectively be used with the excitation energy source **20**.

The excitation energy source **20** may selectively be an ultraviolet light. In the embodiment of FIG. **2**, the lanthanide salt solution may be contained in a squeezable tube **38**. Again, the lanthanide solution can be in several forms, including terbium chloride or europium chloride. In still another embodiment, the lanthanide salt solution is in a sol-gel applied to the set of optical fibers **26**, **28**.

In the device **11** of FIG. **2**, a terbium chloride solution through a tube **37** is injected into the sealed container **12** and photoluminescence from terbium dipicolinate is measured while it is inside the container. The device **11** of FIG. **2** detects dipicolinic acid that may come from bacterial endospores without removing any material from the container **12**. A tube **38** for injecting terbium chloride solution is inserted into the puncture **36** and a forward and backscattering probe **24** having two sets of optical fibers **26**, **28** is also inserted into the puncture. One optical fiber **26** adds UV light to excite photoluminescence, and optical fiber **28** collects photoluminescence for analysis.

The photoluminescence from optical fiber **28** is carried to a collection device **30** (such as an integrating sphere) and a photodetector **32** measures the intensity of the emission with signaling sent from the photodetector to a temporal analyzer **33**. The photodetector (such as a photomultiplier tube in one example) may operate with a narrow band filter **34** (for instance a 540 nm filter). The temporal analyzer **33** (FIG. **2**) and excitation energy source **20** are coupled together and communication is made by an electronic trigger **39**. In the device **11** of FIG. **2**, bacterial endospores may be detected while inside the container without removing the endospores.

Ultraviolet (UV) radiation illuminates the contents of the sealed container **12**. An example of a sealed container **12** is a cardboard box or envelope; an example of a UV source is an arc lamp. The light emitted from the contents is collected by another optical fiber **28** and passed on to an optical collection device. Examples of collection devices **30** would be a lens, an integrating sphere, or a fiber optic.

The light then may selectively pass through a dispersive device **34** that selects a wavelength that is characteristic of the lanthanide salt. An example of a lanthanide can be terbium or europium, an example of a dispersive device **34** is a narrow band filter or a spectrometer, and an example of an emission band is the 542 nm band in terbium salts. A photodetector **32** measures the intensity of emission as a function of time before and after excitation. Examples of photodetectors **32** include photomultipliers, photodiodes and charge coupled diode arrays. The signal from the photodetector is sent to a temporal analyzer **33**. Examples of temporal analyzers: a digital oscilloscope, a multichannel analyzer, and an A/D converter attached to a computer. The temporal analyzer **33** analyzes the temporal profile of the emitted light. An example of such an analysis is the determination of emission lifetime, the measurement of peak intensity, the measurement of time integrated intensity, or phase modulation.

The wavelength dispersive device **34** and the temporal analyzer **33** are employed since many aerosols emit light by photoluminescence. Isolating the terbium dipicolinate emission from the species is important to eliminate false positive detections. As an example, terbium dipicolinate has a strong a narrow emission band a wavelength 542 nm, and in the presence of a surplus of terbium, lifetime of 0.6 ms.

While the principles of the invention have been shown and described in connection with but a few embodiments, it is to be understood clearly that such embodiments are by way of example and are not limiting.

We claim:

1. A method for detecting bacterial endospores inside a sealed container, the method comprising of the steps of:
- puncturing the sealed container;
- injecting a lanthanide salt solution into the container through the puncture;
- inserting two sets of optical fibers into the container;
- exciting the solution with an ultraviolet light using one of the two sets of optical fibers;
- collecting photoluminescence emitted from the excited lanthanide salt solution with a second of the two sets of optical fibers; and
- measuring the photoluminescence from the lanthanide salt in the solution.

2. The method of claim **1** wherein the lanthanide salt is terbium chloride.

3. The method of claim **1** wherein the lanthanide salt is europium chloride.

4. The method of claim **1** wherein the light is transmitted by one of (a) a modulated light source, (b) a pulsed xenon arc lamp, and (c) a pulsed laser.

5. The method of claim **1** further comprising exciting the solution with ultraviolet light using a first optical fiber of a probe having at least two optical fibers.

6. The method of claim **1** further comprising collecting emission from one of the lanthanide salt solution emission bands with a second optical fiber.

7. The method of claim **1** wherein the sealed container is one of: (a) an envelope, (b) a box, and (c) a package.

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